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A SPOT DISEASE OF CAULIFLOWER.

BY

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Plant Industry,
Office of the Chief,
Washington, D. C., May 31, 1911.

Sir: I have the honor to transmit herewith a manuscript entitled "A Spot Disease of Cauliflower," by Miss Lucia McCulloch, scientific assistant in the Laboratory of Plant Pathology, and recommend its publication as Bulletin No. 225 of the series of this bureau.

This paper deals with a disease which is shown to be of bacterial origin and which has not been reported hitherto.

The investigations of this disease have been carried out according to the advice and suggestions of Dr. Erwin F. Smith.

Respectfully,

Wm. A. Taylor,
Acting Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

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INTRODUCTION.

In April and again in May, 1909, diseased cauliflower plants were sent to the Laboratory of Plant Pathology from a farm in southeastern Virginia. In both lots the leaves were closely covered with brownish to purplish gray spots 1 to 3 millimeters in diameter (Pl. I). There were also larger diseased areas due to the coalescing of spots. All parts of the leaves were affected. Where the midribs and veins were badly attacked the tissues had contracted, giving a puckered appearance to the leaves (Pl. II). From the spots a bacterium was secured in pure cultures by means of petri-dish poured plates on agar, and subcultures from colonies thus obtained were used for inoculating healthy cauliflower plants.

The cauliflower heads from the same plants were not in good condition, but no success attended the efforts to secure from them the same kind of bacterium that was found in the leaf spots.

What appears to be the same disease was also received once on cauliflower from Florida.

INOCULATIONS.

All inoculations were made by spraying the plants with pure cultures of the bacterium suspended in water (24 to 48 hour agar slants washed off in sterile water). Young, healthy cauliflower plants 6 to 10 inches high were used, some being kept in infection cages and some merely on a bench in the greenhouse.

The infection shows first on the lower surface of the leaves as sunken water-soaked spots.[1] These are visible on the third day after inoculation. In 4 or 5 days the spots are dark purplish-gray and show on both surfaces. In transmitted light the centers are thin, almost colorless, and surrounded by a dark border. In size they vary from mere points to spots 1.5 millimeters in diameter. In shape they are irregularly angled; the spread of the disease appears to be stopped or hindered by the veins of the leaf. The individual

[1] Some infections from old cultures that had probably lost much of their virulence were first indicated after 6 to 8 days by tiny water-soaked elevations. As the disease progressed the tissues collapsed and a sunken spot resulted.

spots do not become more than 2 to 3 millimeters in largest diameter, though where crowded the spots usually coalesce, forming diseased areas of considerable size.

When only the upper surface of leaves was subjected to the inoculating spray very few infections resulted, while inoculations on the lower surface gave numerous infections (Pl. III). So far as observed in sections the infection takes place only through stomata, and mostly from the lower surface. The older and the very young leaves appear to be partially or even completely immune, while those of intermediate age (on the same plant) may be seriously affected.

The diseased leaves become yellow and fall off in from three to five weeks. The younger leaves and new growth are healthy. Under our rather dry hothouse conditions in no case was there evidence of infection on any but the inoculated leaves.

From spots developing on leaves inoculated with the original culture the organism was repeatedly isolated by means of petri-dish poured plates, and subcultures from colonies were again used for inoculations, always with the production of the characteristic infection.

The checks in all cases remained healthy; also numerous other cauliflower plants in the same greenhouse.

Cabbages inoculated with this organism became infected in the same manner as the cauliflower with one exception, viz, the spots were darker. From spots appearing on the inoculated cabbages the organism was isolated and tested on cauliflower, with the result of the production of the disease.

During May and June of 1909 all inoculations resulted in successful and typical infections.

On July 10, 1909, seven plants were inoculated in the usual manner and, contrary to expectations, no infections resulted. The conditions were the same as in previous inoculations, except that the temperature was higher at this time, 26° to 34° C. (78° to 93° F.). Subsequent experiments show that the bacterium causing the disease refuses to grow in artificial media at 29° C. (84° F.) or above. It is probable, therefore, that in this case the high temperature prevented infection.

After July no more plants were available for inoculation until January 24, 1910, when 10 plants were inoculated. No infections resulted. On February 2, 1910, 10 more plants were inoculated and no infections resulted. All conditions seemed favorable, and loss of virulence in the culture was suspected as the cause of failure to infect. The cultures used for inoculation were descended through numerous transfers made during the winter. An agar-stock culture which had not been transferred since September, 1909, was then tried. Fresh agar-slant cultures were made from the September stock and used

when 24 hours old (February 12, 1910) for inoculating cauliflower plants. This time infections resulted. The infections were very slight, however, and the spots too few in number to cause any noticeable injury to the plants, but the bacteria plated from these spots produced typical colonies on agar and characteristic growth in artificial media. Cauliflower plants inoculated with this new strain became very generally infected, which fact seems to indicate that the organism had increased in virulence by passage through the host.

On December 6, 1910, cabbage, cauliflower, turnip, rutabaga, radish, and mustard were inoculated in cages by spraying with sterile water to which had been added agar-streak cultures 3 days old. All of these plants were young. There were two to four of each sort. The material for inoculation was obtained from a cauliflower inoculated November 22, 1910. Infections were obtained on cabbage and cauliflower (six days), but not on the other plants. Sections of the spots made on the tenth day showed them to be full of bacteria.

On March 4, 1911, three cauliflower plants were again inoculated by spraying with sterile water to which had been added 2-day-old agar-slant cultures. These plants were about 8 inches high and very healthy. They were kept in an infection cage for two days. At the end of 10 days there were very small dark specks on each of the plants. These specks were in the center of a small semitransparent elevation. A microscopic examination showed bacteria present in these spots. The flower stalks of plant 104 also showed elongated water-soaked spots, darker in the center. These also contained bacteria and plates made from them yielded the organism in pure culture. The flowering part of plant 104 bore no spots, although it had been drenched with the spray. The check plant remained healthy.

Several attempts to inoculate the heads of the cauliflower gave no satisfactory results. Growing heads were copiously inoculated and kept under moist conditions, but no infections occurred. Infection spots similar to those on leafstalks and midribs occurred on some of the larger stalks of the flower head, while the flowering parts remained free from infection. Mature heads from the market were also inoculated, but as decay of the tender surface was general in the checks as well as in the inoculated heads, the results are not conclusive.

DESCRIPTION OF THE CAULIFLOWER LEAF-SPOT ORGANISM.

MORPHOLOGY.

The organism is a short rod, forming long chains in some media. Ends rounded. Size from leaf 1.5 to 2.4 μ by 0.8 to 0.9 μ. Size in 24-hour beef-agar culture, temperature 20° to 25° C., 1.5 to 3 μ by 0.9 μ. No spores are produced. The organism is actively motile by

means of one to five polar flagella, which are two to three times the length of the rod. (Stained by Van Ermengem's method; also by Hugh Williams's method.) Motility occurs in most artificial media. In beef-bouillon cultures grown and kept at 0.5° to 1.5° C. for four months the organism is still motile. Involution forms were found in alkaline beef bouillon (−17 on Fuller's scale). Pseudozoogloeæ occur in Uschinsky's solution and in acid beef bouillon.

REACTION TO STAINS.

The organism does not stain by Gram. Modified Gram, using amyl alcohol, gives a deep blue stain. It stains readily and strongly with carbol fuchsin, with an alcoholic solution of gentian violet, and with a stain obtained from Dr. Kinyoun which contains methylene blue, silver nitrate, azure I, and azure II. It is not acid fast.

CULTURAL CHARACTERS.

Agar plates (+15 peptonized beef bouillon with 1 per cent agar).—The colonies are visible on the second day as tiny white specks (temperature 23° C.).

In three to four days the colonies are 1 to 3 millimeters in diameter, white (opalescent in transmitted light), round, smooth, flat, shining, and translucent, with edges entire. Structure, under hand lens, coarsely granular with internal reticulations. Buried colonies small, lens shaped. With age the colonies become dull to dirty white, slightly irregular in shape, the edges undulate, slightly crinkled, and with indistinct radiating marginal lines. The internal reticulations disappear and the coarsely granular appearance changes to finely granular. In thinly sown plates 7-day-old colonies are 6 to 8 millimeters in diameter; 15-day-old colonies are 12 to 15 millimeters in diameter.

Agar stabs.—The surface of the agar is covered in two days (22° to 24° C.) by a thin white growth. For several days the stab shows a moderate growth in the upper 8 to 10 millimeters, but this does not continue. Finally, the stab is almost, if not quite, invisible. Crystals appear in the stab and on the surface.

Agar slants.—In smear cultures the surface is covered in two days (temperature 19° to 21° C.) with a thin white growth, glistening, coarsely and irregularly pitted. White sediment in the V.

In streak cultures in two days (temperature 19° to 21° C.) the streak is 3 to 5 millimeters wide, white, margins slightly undulate. The internal reticulations seen in colonies on plates are present in the streak cultures. At right angles to the streak are fine lines extending from center to margin.

Agar cultures become slightly greenish.

Beef bouillon.—Peptonized beef bouillon +15 held at 24° to 25° C., if inoculated from young, vigorous bouillon cultures, clouds thinly in 6 hours and is moderately to heavily clouded in 24 hours. The growth is best at the surface, where a white layer is formed. This is not a true pellicle, as it disintegrates when the cultures are handled.[1] No zoogloeæ are present. There is no rim. In two days there are heavy clouds and a moderate amount of white flocculent precipitate. After several weeks the precipitate is white and slimy, moderate in quantity, and with small crystals in it. The medium becomes slightly greenish. After several months the precipitate is viscid.

Acid bouillon.—In neutral beef bouillon plus vegetable acids, growth occurs until an acidity of +34 for oxalic acid and +36 for malic and citric acids (Fuller's scale) is reached. There is no rim or pellicle. Occasionally pseudozoogloeæ are formed in the more acid media.

Microscopic examination shows most of the organisms greatly reduced in length, some so short as to be spheroidal. That these were not contaminations was proved by plating out and by tests on other culture media.

Alkaline bouillon.—In alkaline beef bouillon (NaOH used) the organism grew well in −17, −19, −22, less in −23, and not at all in −25, −26, and −28 (Fuller's scale). Involution forms and filaments were present in −17 beef bouillon when two weeks old.

Bouillon with sodium chlorid.—In beef bouillon plus 2 per cent NaCl the growth is as good as in plain beef bouillon. With the addition of more NaCl the growth gradually lessens until it is scarcely noticeable in a 5 per cent solution. When grown in a 4 per cent solution, the organism is not motile. In a 2 per cent solution the organism is motile, but less so than in beef bouillon without NaCl.

Beef bouillon over chloroform.—For the first 24 hours the growth is somewhat retarded. By the end of 48 hours no difference could be seen between cultures over chloroform (5 c. c. of chloroform with 10 c. c. of beef bouillon not shaken) and those in plain beef bouillon.

Loeffler's blood serum.—Growth of stroke is moderate, smooth, shining; color creamy; margins finely crinkled. No liquefaction. After three months the whole medium was slightly browned.

Cohn's solution.—Moderate clouding and white precipitate; no rim, pellicle, or zoogloeæ; no fluorescence. After some weeks feather-like crystals of considerable size (5 to 10 by 2 to 6 mm.) are formed.

Fermi's solution.—Moderate clouding at first. Precipitate moderate to abundant, white, flocculent. Pellicle white, tender, sinking in strings and masses. Finally the medium is densely clouded and

[1] Old cultures kept for several months at 0.5° to 1.5° C. had a delicate pellicle.

pale green-fluorescent (between water green and greenish glaucous, Repertoire de Couleurs, Paris, 1905); more precipitate than in beef bouillon.

Nutrient gelatin (+10 on Fuller's scale).—The stab cultures liquefied in 8 to 10 days (temperature 17° to 18° C.). Growth from surface crateriform. Slight, white, granular precipitate. Slight green fluorescence.

The plate cultures showed no signs of growth in 24 hours at 17° to 18° C. In three days well-isolated colonies vary from mere points to round growths 2 millimeters in diameter. The gelatin is liquefied in cuplike hollows. Margin of smaller colonies entire, of larger colonies fimbriate. Thickly sown plates entirely liquefied in two days at 15° to 16° C.

Litmus milk.—The medium becomes dark blue at the surface in 12 to 24 hours. The darkening proceeds downward in definite layers until in 8 to 10 days the whole medium is dark blue with a slight white precipitate. During six months' observation the medium remained dark blue (reflected light) and liquid. Finally by evaporation the medium becomes thickened, but there is at no time any separation into curd and whey.

A few cultures showed a trace of reduction of litmus at the bottom.

Milk.—As in the litmus-milk cultures, growth and color-change in the milk begin at the surface, proceeding downward in definite layers. In 15 to 20 days the whole tube (10 c. c. of milk) is yellow (near Ridgway's Naples yellow, but somewhat duller and with a greenish tinge) and translucent. No separation into curd and whey. Fat not changed. In four months the medium is quite dark (reddish-brown) and somewhat thick (evaporated to about 5 c. c.). Small tyrosin crystals are formed. These are distinctly visible only with a lens.

Uschinsky's solution.—Growth moderate to copious; pellicle white, tender, breaking and sinking easily. Pseudozoogloeæ are present. There is a greenish fluorescence. The old cultures are much like those in Fermi's solution.

OTHER CHARACTERISTICS.

Fermentation tubes.—The organism is aerobic and does not form gas. It was tested in fermentation tubes in the presence of dextrose, saccharose, lactose, maltose, glycerin, and mannit, each of these carbon compounds being added to a basal solution consisting of 1 per cent of Witte's peptone dissolved in water. It did not grow in the closed end of the fermentation tubes in the presence of any of these substances.

Ammonia production.—Moderate.

Nitrates.—Nitrates are not reduced.

Indol.—Indol production is feeble.

Hydrogen sulphid.—Hydrogen sulphid is not formed in cultures on beef-peptone agar, potato cylinders, turnip cylinders, or in beef bouillon or milk.

TEMPERATURE RELATIONS.

Thermal death point.—The thermal death point is 46° C. The following tests were made: Newly inoculated beef-bouillon (+15) cultures in tubes were suspended in a hot-water bath where they were kept for 10 minutes at a constant temperature, then removed to room temperature (20° to 24° C.). First, temperatures ranging from 40° to 50° C. were tried, and, the thermal death point seeming to lie about halfway between, trials were again made of 45°, 46°, and 47° C. More than half of the cultures exposed to 45° C. for 10 minutes clouded in 3 to 5 days. Of cultures exposed to 46° C. 1 out of 12 clouded after 11 days. The others never clouded. Of 20 cultures exposed to 47° C., none clouded.

Optimum temperature.—The optimum temperature for growth is 24° to 25° C.

Maximum temperature.—The maximum temperature for growth is very low, viz, 29° C.

Minimum temperature.—The minimum temperature for growth is below 0° C.

The organism was dead after exposure for 3½ days at 33° to 36° C. in beef bouillon.

EFFECT OF DESICCATION.

When young, well-clouded beef-bouillon cultures were dried on cover glasses and kept in a dark place at temperatures of 22° to 25° C., 75 per cent were killed in 24 hours and 90 per cent in 48 hours. All were dead in five days.

EFFECT OF SUNLIGHT.

Four minutes' exposure to sunlight killed all organisms in thinly sown agar poured plates exposed bottom up on ice, one-half of each plate being covered as a check.

EFFECT OF FREEZING.

Freezing by means of salt and pounded ice for two and five hours in +15 beef bouillon had no effect in reducing the number or the vitality of the organisms, as shown by poured-plate cultures made before and after freezing.

Beef bouillon (10 c. c.) inoculated and at once frozen and kept at temperatures of −4° to −18° C. (average −12° C.) for nine days

showed no growth during this period, but clouded moderately three days after removal to temperature of 18° to 20° C., and plates from this tube gave pure cultures of the cauliflower organism. Plates poured before and after 10 days' freezing showed considerable reduction in the numbers of organisms, but the growth of the living ones was not retarded. Some tubes of beef bouillon, inoculated with a 3-millimeter loop from a 48-hour bouillon culture and kept frozen for seven days, clouded within 48 hours after removal to a temperature of 18° to 20° C. Another frozen for 22 days did not cloud after removal to temperatures of 18° to 20° C. The check clouded. The organism grows readily at low temperatures, e. g., beef-bouillon cultures clouded in seven days when kept at temperatures of 0° to 1° C.

VITALITY ON CULTURE MEDIA.

This organism remains alive for six to eight months at temperatures varying from 18° to 24° C. on beef agar, Loeffler's blood serum, and potato cylinders, and in peptonized beef bouillon (+15), beef gelatin (+10), milk, Uschinsky's, Fermi's, and Cohn's solutions. Evaporation was not prevented in these cultures and the media became concentrated, often dry, and yet the organism was frequently alive. Beef-agar and beef-gelatin cultures at temperatures of 12° to 15° C. and subject to less evaporation (in refrigerator) were dead after eight months. In media less favorable for the growth of this organism, as beef bouillon plus salt, alkali, or acid, the bacteria live but two to three months.

GROUP NUMBER.

The group number, according to the descriptive chart of the Society of American Bacteriologists, is 211.3332023.

NAME OF ORGANISM.

This organism appears to be an undescribed form, and because of the characteristic spotting of the affected leaves the name *Bacterium maculicolum* (n. sp.) is suggested.

LATIN DIAGNOSIS.

BACTERIUM MACULICOLUM (N. SP.).

Baculis in hospite brevibus, cylindricis, apicibus rotundatis, solitariis, saepe binis (in agar-agar quandoque 10–30 baculis in filamenta conjunctis); baculis 1.5–3.0 μ x 0.8–0.9 μ; 1–5 flagellis polaribus mobilibus; aerobiis, asporis.

Habitat in foliis vivis Brassicae oleraceae in maculis 1–3 mm. latis, purpureo-griseo colore. Coloniae gelatinam liquefacientes. Coloniae in agar-agar rotundae, albae, nitentes. Si baculi in petri-vasibus rare seruntur, in septimo die colonae 6–8 mm. diam. sunt. Baculi

methodo Gram non colorantur. Nitrum non redigitur. Lac,sterile alcalinum fit; initio translucidum, flavum pallidum demum opacum, brunnen et gelatum; casein non segregatur. Inter temperaturam 29° C. et temperaturam −5° C. culturae crescunt. Si culturae novae in infusione carnis ¼ horam in temperatura 46° C. tenentur, moriuntur. Inter temperaturas −5° C. et −15° C. per decem dies non moriuntur. Si baculi siccantur vel soli exponuntur, celeriter moriuntur. In foliis vivis Brassicae oleraceae aspergendo inoculatis, maculae in 3–4 diebus producuntur. Contagium in stomatibus fit.

SUMMARY.

The leaf-spot disease of cauliflower described in the preceding pages is due to a bacterial organism, which was secured in pure culture from the leaf spots and inoculated into healthy cauliflower plants, with production of the disease. Healthy cabbages inoculated with the organism also showed similar infection.

Inoculations during July, 1909, were unsuccessful because of the higher temperature.

The heads of cauliflower gave no satisfactory results when inoculated with the organism.

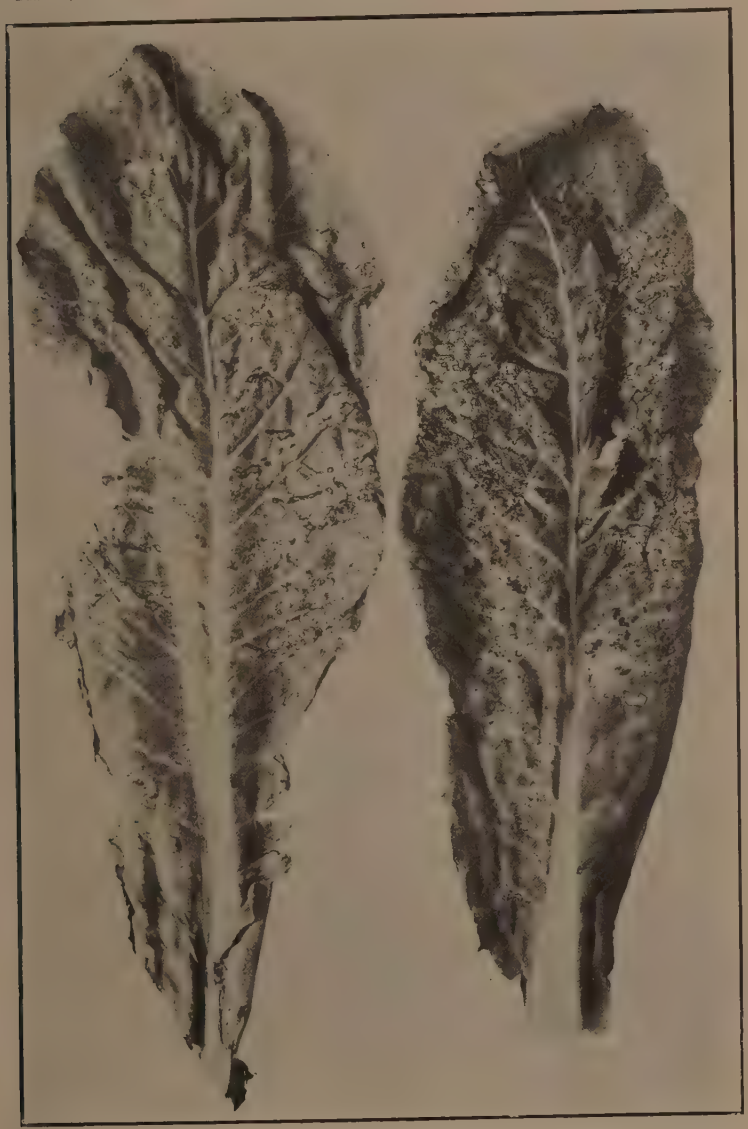
The name *Bacterium maculicolum* has been suggested for this organism. It is a schizomycete, pathogenic to crucifers, causing numerous small spots on cauliflower and cabbage. Entrance by way of the stomata. Organism white, but causing a greenish fluorescence in some media (beef bouillon +15, beef gelatin +10, beef agar +15, Uschinsky and Fermi). Motile by means of one to several polar flagella. A short rod (1.5 to 3 μ by 0.9 μ), single or in chains in some media (10 to 30 μ long on agar; 10 to 24 μ long in beef bouillon plus 4 per cent sodium chlorid). Does not stain by Gram; stains deeply with amyl Gram.

No spores. Involution forms (found in alkaline beef bouillon) and pseudozoogloeæ. Aerobic. Liquefies gelatin slowly. Does not liquefy Loeffler's blood serum. Not gas forming. Feeble production of ammonia, indol, and hydrogen sulphid. Nitrates not reduced. Tolerates acids, citric and malic to +36 and oxalic to +34 (Fuller's scale). Tolerates sodium hydroxid in beef bouillon to −25 (Fuller's scale).

Optimum temperature 24° to 25° C. Thermal death point 46° C. Will not grow in beef bouillon (+15) or on agar (+15) at 29° C. Grows at 0° C. and below. Grows well in bouillon over chloroform. Grows in Cohn's solution. Blues litmus milk.

The most striking facts about the organism are its ability to grow at temperatures below freezing and its failure to grow at a common summer temperature (85° F.).

The leaves of the attacked plants fall off.



UPPER SURFACES OF CAULIFLOWER LEAVES FROM VIRGINIA, SHOWING NATURAL INFECTION WITH BACTERIUM MACULICOLUM. PHOTOGRAPHED MAY 4, 1909.

UNDER SURFACES OF CAULIFLOWER LEAVES FROM VIRGINIA, SHOWING MIDRIBS SPOTTED BY NATURAL INFECTION WITH BACTERIUM MACULICOLUM. PHOTOGRAPHED MAY 4, 1909.

UNDER SURFACES OF CAULIFLOWER LEAVES FROM HOTHOUSE; INOCULATED BY SPRAYING ON MAY 19, 1909, WITH BACTERIUM MACULICOLUM. PHOTOGRAPHED JUNE 2, 1909.